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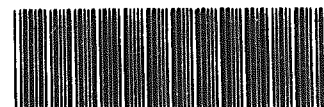
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Office of Toxic Substances
U.S. Environmental Protection Agency
401 M Street, S.W.
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ATTN: 8(e) Coordinator

Contains No CBI

Dear Sir:

SUBJECT: ACUTE TOXICITY OF C₁₈ SECONDARY ALCOHOL SULFATE, SODIUM SALT (CAS # 6858-55-5), TO THE FATHEAD MINNOW AND DAPHNIA MAGNA

The subject TSCA 8(e) submission was filed by Shell Oil Company January 7, 1994 and preliminary data transmissions were provided. The complete reports (attached) are now available and are provided as supplemental information to the original TSCA 8(e) filing.

These supplemental reports are filed to provide information EPA may find useful. In no way are they intended as a waiver of any rights or privileges belonging to Shell Oil Company as the reporting corporation, its agents or employees. The reporting corporation, its agents and employees, reserve the right to object to these reports' use or admissibility in any subsequent judicial or administrative proceeding against the corporation, its agents or employees.

These reports have been compiled based on information available as of the date of filing. The corporation, its agents and employees reserve the right to supplement the data contained in these reports, and to revise and amend any conclusions drawn therefrom.

These reports contain no confidential business information.

The following person should be contacted if you have questions or a need for discussion.

SH9428303.WPD

J. C. Willett
Manager, Product Safety and Compliance
Shell Oil Company
P. O. Box 4320
Houston, TX 77210
Telephone No. 713-241-6958
Fax. 713-241-3325

Very truly yours,

A handwritten signature in dark ink, appearing to read "R. N. Shulman". The signature is fluid and cursive, with a long horizontal stroke extending to the right.

R. N. Shulman, General Manager
Health, Safety, and Environment
Shell Oil Company

THG/sjh

Attachments

DAN 218:
A 96-HOUR STATIC-RENEWAL ACUTE TOXICITY TEST
WITH THE FATHEAD MINNOW (*Pimephales promelas*)

FINAL REPORT

WILDLIFE INTERNATIONAL LTD. PROJECT NUMBER: 109A-111

TITLE 40 OF THE CODE OF FEDERAL REGULATIONS
PART 797.1400

AUTHORS:

Kurt R. Drottar
James P. Swigert, Ph.D.

Contains No CBI

STUDY INITIATION DATE: December 15, 1993

STUDY COMPLETION DATE: August 26, 1994

Submitted to

Shell Development Company
Westhollow Technology Center
3333 Highway Six South
Houston, Texas 77082



WILDLIFE INTERNATIONAL LTD.



8598 Commerce Drive
Easton, Maryland 21601
(410) 822-8600

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GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

SPONSOR: Shell Development Company

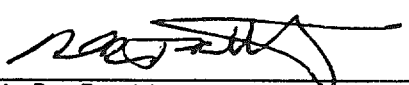
TITLE: DAN 218: A 96-Hour Static-Renewal Acute Toxicity Test
with the Fathead Minnow (*Pimephales promelas*)

WILDLIFE INTERNATIONAL LTD. PROJECT NUMBER: 109A-111

STUDY COMPLETION DATE: August 26, 1994

This study was conducted to conform with Good Laboratory Practice Standards as published by the U.S. Environmental Protection Agency in 40 CFR Part 792, 17 August 1989, with the following exception:

Analytical measurements were not performed to confirm exposure concentrations. Analyses of this type must be performed manually due to lack of repeatability when using an automated system. After discussion with the Sponsor, it is believed that this poor repeatability is due to adsorption of the surfactant on the teflon lines of the automated system. Due to the prohibitive amount of time involved to perform the analyses, the decision was made by the Sponsor not to perform the work given the fact that analyses had already been provided in support of the DAN 214 and 216 compounds.

STUDY DIRECTOR:
Kurt R. Drott
Senior Aquatic Biologist

DATE: 8/26/94

REPORT APPROVED BY:
James P. Swigert, Ph.D.
Manager, Aquatic Toxicology

DATE: 8/26/94

Sponsor

DATE: _____

Applicant/Submitter

DATE: _____

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QUALITY ASSURANCE STATEMENT

This study was examined for conformance with Good Laboratory Practice Standards as published by the U.S. Environmental Protection Agency in 40 CFR Part 792, 17 August 1989. The dates of all inspections and audits and the dates that any findings were reported to the Study Director/Laboratory Management were as follows:

ACTIVITY	DATE CONDUCTED	DATE REPORTED TO STUDY DIRECTOR/MANAGEMENT:
Protocol	January 4, 1994	January 4, 1994
Test substance preparation	January 4, 1994	January 4, 1994
Test Initiation	January 4, 1994	January 4, 1994
Data and Draft Report	February 21-22, 1994	February 23, 1994
Second Draft Report	July 22, 1994	July 22, 1994
Final Report	August 23, 1994	August 23, 1994

Lisa Read
Lisa Read
Quality Assurance Representative

DATE

8/26/94

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SUMMARY

SPONSOR:	Shell Development Company Westhollow Technology Center 3333 Highway Six South Houston, Texas 77082
CONTACT:	Ms. Diana C. L. Wong
LOCATION OF STUDY, RAW DATA AND FINAL REPORT:	Wildlife International Ltd. Easton, Maryland 21601

WILDLIFE INTERNATIONAL LTD. PROJECT NUMBER:	109A-111
TEST SUBSTANCE:	DAN 218
STUDY:	DAN 218: A 96-Hour Static-Renewal Acute Toxicity Test with the Fathead Minnow (<i>Pimephales promelas</i>)
NOMINAL TEST CONCENTRATIONS:	Negative Control, 0.052, 0.086, 0.14, 0.24 and 0.40 mg DAN 218/L
TEST DATES:	Experimental Start - January 4, 1994 Experimental Termination - January 8, 1994
LENGTH OF TEST:	96 Hours

96-HOUR LC50:	0.20 mg DAN 218/L
95% CONFIDENCE LIMITS:	0.18 and 0.23 mg DAN 218/L
NO MORTALITY CONCENTRATION:	0.086 mg DAN 218/L

TEST ORGANISM:	Fathead Minnow (<i>Pimephales promelas</i>)
SOURCE OF TEST ORGANISMS:	Wildlife International Ltd. Easton, Maryland 21601
AGE:	Juveniles
WET WEIGHT:	Mean=0.66 g; Range=0.38 g to 0.90 g (10 Controls)
STANDARD LENGTH:	Mean=34 mm; Range=30 mm to 37 mm (10 Controls)

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INTRODUCTION

This study was conducted by Wildlife International Ltd. for Shell Development Company, at the Wildlife International Ltd. aquatic toxicology facility in Easton, Maryland. The test was conducted from January 4, 1994 to January 8, 1994. Raw data generated by Wildlife International Ltd. and an authenticated copy of the final report are filed under Project Number 109A-111 in archives located on the Wildlife International Ltd. site.

OBJECTIVE

The objective of this study was to evaluate the acute toxicity of DAN 218 to the fathead minnow (*Pimephales promelas*) during a 96-hour exposure period under static-renewal test conditions.

EXPERIMENTAL DESIGN

Fathead minnows were exposed to a geometric series of five test concentrations and a negative (well water) control under static-renewal test conditions for 96 hours. Two replicate test chambers were maintained in each treatment and control group, with 10 fish in each test chamber. Nominal test concentrations were selected in consultation with the Sponsor, and were based upon the results of a range finding test. Nominal test concentrations selected were 0.052, 0.086, 0.14, 0.24, and 0.40 mg DAN 218/L. Analytical measurements were not performed to confirm exposure concentrations. The test results are presented in terms of nominal concentrations throughout this report.

Immediately prior to initiating the test, fish were impartially removed from holding tanks in groups of two and distributed among the test chambers until each contained 10 fish. At approximately 24, 48 and 72 hours, new test solutions were prepared and fish were transferred to the new solutions. Observations of mortality and other clinical signs of

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toxicity were made at approximately 7, 24, 48, 72, and 96 hours after test initiation. Cumulative percent mortality values in each treatment group were used to calculate LC50 values and 95% confidence limits at 24, 48, 72, and 96 hours. The no mortality concentration was determined by visually examining the mortality data.

MATERIALS AND METHODS

The study was conducted according to the procedures outlined in the protocol, DAN 218: A 96-Hour Static-Renewal Acute Toxicity Test with the Fathead Minnow (*Pimephales promelas*). The protocol was based on procedures outlined in TSCA Title 40 of the Code of Federal Regulations, Part 797.1400 (1), and ASTM Standard E-729-88, Standard Practice for Conducting Acute Toxicity Tests with Fishes, Macroinvertebrates and Amphibians (2).

Test Substance

The test substance was received from the Shell Development Company on November 5, 1993. The test substance was a white powder identified as: WRC TOX SAMPLE NO. 1233, PSIS 1232-3, STORAGE COND. Ambient, COMPOSITION DAN 218 Secondary alkyl sulfonate, NaOH, Na₂SO₄, HAZARDS see accompanying PSIS for hazard information, DATE DISPENSED 3 NOV. 1993. RESPONSIBLE INDIVIDUAL H.C. SMITH/EC-644, EXPIR. DATE NOV. 1994, DAN 218, 350 g, DEVELOPMENTAL ANIONIC DAN 200 SERIES. The test substance was assigned Wildlife International Ltd. Identification Number WIL-2762 upon receipt. Information provided by the Sponsor indicated a purity of 88% and that the test substance was incorrectly labeled (secondary alkyl sulfonate should have been secondary alkyl sulfate).

Preparation of Test Concentrations

All glassware used to make the stock solutions was serially rinsed with reverse osmosis (RO) water, 70% isopropanol/30% water, and RO water

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prior to use. The primary stocks were prepared by adding the appropriate amount of test substance to RO water to achieve a concentration of 95 mg DAN 218/L. The stocks were heated to approximately 60°C and inverted to aid in solubilization of the test substance. New primary stock solutions were freshly prepared on Days 1, 2 and 3 of the test for renewal of test solutions. The test solutions were prepared by adding the appropriate amount of primary stock to dilution water in the test chambers. The test solutions were mixed by stirring with a glass rod for approximately 1 minute. Stock concentrations and the resultant test concentrations were not adjusted for the purity of the active ingredient in the test substance (88%).

Test Organism

The fathead minnow (*Pimephales promelas*) was selected as the test species for this study. The fathead minnow was considered representative of an important group of aquatic vertebrates and was selected for use in this test based upon past history of use, and ease of culturing in the laboratory. Fish used in the test were juveniles obtained from cultures maintained by Wildlife International Ltd., Easton, Maryland.

The juvenile fathead minnows were held in cultures for at least 14 days prior to testing. The water used during holding was obtained from the same source as water used during the test. The test fish were acclimated to test conditions for approximately 49 hours prior to test initiation. No mortalities occurred during acclimation, and the fish showed no signs of disease or stress.

All fish used in the test were from the same source and year class. The lengths and weights of 10 control fish were measured at the end of the test, and it was determined that the standard length of the longest fish measured was no more than twice the standard length of the shortest. The average length was 34 mm with a range of 30 mm to 37 mm, while the average weight was 0.66 g with a range of 0.38 g to 0.90 g. Instantaneous loading

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in the test chambers was calculated to be 0.44 grams of fish per liter of test water.

During holding, the fish were fed flaked fish food (Zeigler Brothers, Inc., Gardners, PA 17324) live *artemia* nauplii (Artemia Inc., Newark, CA 94560) and frozen brine shrimp (Kordon, Hayward, CA 94545). Feed was withheld during acclimation and the test.

Test Apparatus

The test chambers were Teflon® lined 25-L polyethylene tanks containing 15 L of test solution. The test chambers were impartially positioned in an environmentally controlled room designed to maintain a temperature of $22 \pm 1^\circ\text{C}$. The test chambers were labeled with the project number, test concentration, and replicate.

Dilution Water

The water used for holding, acclimation, and testing was freshwater obtained from a well 45 meters deep located on the Wildlife International Ltd. site. The well water is characterized as medium-hard water. The conductivity, hardness, alkalinity, and pH of the well water during the four-week period immediately preceding the test are presented in Appendix I.

The well water was passed through a sand filter to remove particles greater than approximately 25 μm , and pumped into a 37,800-L storage tank where the water was aerated with spray nozzles. Prior to delivery to the diluter system, the water again was filtered to remove microorganisms and particles. The results of analyses performed to measure the concentrations of pesticides and metals in well water used by Wildlife International Ltd. are presented in Appendix II. The dilution water was aerated overnight prior to use in the test.

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Environmental Conditions

Lighting used to illuminate the cultures and test chambers during holding, acclimation, and testing was provided by fluorescent tubes that emitted wavelengths similar to natural sunlight (e.g., Chroma 50). A photoperiod of 16 hours of light and 8 hours of darkness was controlled with an automatic timer. A 30-minute transition period of low light intensity was provided when lights went on and off to avoid sudden changes in lighting. Light intensity during the test was approximately 323 lux at the surface of the water.

Temperature was measured in both old and new solutions of all replicate test chambers at approximately 24-hour intervals using a calibrated hand-held thermometer. Temperature also was measured continuously in one negative control replicate using a Fulscope ER/C Recorder. The target test temperature during the study was $22 \pm 1^\circ\text{C}$. The pH and dissolved oxygen content of the water in both the old and new test solutions of all replicate test chambers were measured at approximately 24-hour intervals. Hardness, alkalinity, acidity, total organic carbon (TOC), particulate matter and conductivity were also measured in the dilution water.

Measurements of pH were made using a Fisher Accumet Model 915 pH meter, and dissolved oxygen was measured using a Yellow Springs Instrument Model 51B dissolved oxygen meter. Total organic carbon was measured with a Shimadzu Model 5000 TOC analyzer. Conductivity was measured using a Yellow Springs Instruments Model 33 Salinity-Conductivity-Temperature meter. Hardness, acidity, particulate matter and alkalinity measurements were made in accordance with *Standard Methods for the Examination of Water and Wastewater* (3).

Observations

All organisms were observed at approximately 7, 24, 48, 72 and 96 hours to evaluate the number of mortalities and the number of individuals

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exhibiting clinical signs of toxicity and abnormal behavior. The test organisms were transferred from the holding tanks into the test solutions and from old test solutions to new test solutions with an aquarium net. Dead organisms were removed from the test chambers every 24 hours. At test termination, 10 control fish were retained for measurements of length and weight.

Statistical Analyses

The data were analyzed using the computer program of C. E. Stephan (4). The program was designed to calculate the LC50 value and the 95% confidence interval by probit analysis, the moving average method, or binomial probability with nonlinear interpolation (5, 6, 7). In this study, the binomial method was used to evaluate mortality at 24 hours. The 48, 72 and 96-hour LC50's were calculated using the moving average method. The no mortality concentration was determined by visual inspection of the mortality data.

Analytical Chemistry

Water samples were collected in each treatment and control: 1) at test initiation; 2) on Days 1, 2 and 3 in old and new solutions and 3) at test termination. If 100 percent mortality occurred in any treatment, then collection of water samples was terminated after the next sampling interval. All water samples were collected from both replicates of each treatment and control group and composited for analysis. Samples were collected in glass containers with Teflon®-lined caps. Sample containers were rinsed with RO water, 70% isopropanol/30% water, and RO water prior to use. Samples of each primary stock were also collected. All samples were preserved with 1 percent formalin, pH adjusted to approximately 8.0 using NaOH and shipped to Shell Development Company, Houston, TX for analysis.

Attempts at DAN 218 analysis by the Sponsor using the automated extraction system for DAN 214 and DAN 216 analyses yielded poor and

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inconsistent recoveries (less than 70%). Although manual extraction of spiked samples at the 50 $\mu\text{g/L}$ level have yielded 80 to 85% recoveries, a prohibitive amount of time would have been required to analyze all the DAN 218 samples manually. Therefore, the Sponsor requested that the analytical portion of the study be discontinued and the samples were not analyzed to confirm exposure concentrations.

RESULTS AND DISCUSSION

Observations and Measurements

Measurements of temperature, dissolved oxygen, and pH are presented in Tables 1, 2 and 3, respectively. Temperatures were within the limits of the range established for the test, or $22 \pm 1^\circ\text{C}$. Dissolved oxygen concentrations exceeded 60% of saturation throughout the test. Measurements of pH during the test ranged from 8.2 to 8.6.

Daily observations of mortality and other signs of toxicity used to evaluate the effects of the test substance on fathead minnows are shown in Table 4. Test organisms in the control, 0.052 and 0.086 mg DAN 218/L treatment groups appeared normal and healthy during the test. In the 0.14 and 0.24 mg DAN 218/L treatment groups, mortality was 15% and 65% at test termination, respectively. In the 0.40 mg DAN 218/L treatment group, mortality was 5% at 7 hours and 100% at 24 hours. LC50 values and 95% confidence limits for the 24, 48, 72 and 96-hour time period were calculated from the mortality data (Table 5 and Appendix III).

CONCLUSION

The 96-hour LC50 value for fathead minnows exposed to DAN 218 was 0.20 mg DAN 218/L. The lower and upper 95% confidence limits were 0.18 and 0.23 mg DAN 218/L, respectively. The no mortality concentration was 0.086 mg DAN 218/L.

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REFERENCES

- 1 Title 40 of the Code of Federal Regulations, Part 797, Section 1400, Fish Acute Toxicity Test. July 1992.
- 2 American Society for Testing and Materials. 1988 Standard Practice for Conducting Acute Toxicity Tests with Fishes, Macroinvertebrates, and Amphibians, ASTM E 729-88. Philadelphia, PA.
- 3 APHA, AWWA, WPCF, Standard Methods for the Examination of Water and Wastewater. 16th Edition, 1985. American Public Health Association, Washington, D.C.
- 4 Stephan, C.E. U.S. EPA, Environmental Research Laboratory, Duluth, Minnesota. 1978.
- 5 Finney, D.J. Statistical Methods in Biological Assay, second edition. Griffin Press, London. 1971.
- 6 Thompson, W.R. Bacteriological Reviews, Vol. II, No. 2. June, 1947. Pp. 115-145.
- 7 Stephan, C.E. "Methods for Calculating an LC50", Pages 65-81, in Aquatic Toxicology and Hazard Evaluations, American Society for Testing and Materials. Publication Number STP 634 (1977), pp 65-84. Philadelphia, PA.

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TABLE 1

Temperature of Water in the Test Chambers

Sponsor: Shell Development Co.									
Test Substance: DAN 218									
Test Organism: Fathead Minnow (<i>Pimephales promelas</i>)									
Dilution Water: Well Water									
Nominal Concentration (mg DAN 218/L)	Replicate	Temperature (°C)							
		Day 0 ¹ (New)	Day 1 (Old)	Day 1 (New)	Day 2 (Old)	Day 2 (New)	Day 3 (Old)	Day 3 (New)	Day 4 (Old)
Control	A ²	22.5	22.1	22.0	22.0	22.2	22.2	22.1	21.8
	B	22.4	22.1	22.1	22.0	22.2	22.2	22.2	21.9
0.052	A	22.4	22.1	21.9	21.9	22.2	22.2	22.0	21.9
	B	22.5	22.1	22.0	22.0	22.3	22.2	22.0	22.0
0.086	A	22.5	22.2	22.0	22.0	22.1	22.2	22.0	21.9
	B	22.5	22.0	22.0	21.9	22.3	22.2	22.0	21.8
0.14	A	22.2	22.0	21.6	21.4	22.1	22.2	22.0	21.8
	B	21.8	21.6	21.6	21.4	22.1	21.6	21.5	21.8
0.24	A	22.4	21.9	22.1	22.0	22.1	22.1	22.3	21.8
	B	22.4	21.9	22.1	22.1	22.2	22.2	22.2	21.8
0.40	A	22.5	22.1	- ³	-	-	-	-	-
	B	22.5	22.2	-	-	-	-	-	-

¹The 0-hour dilution water measurements for hardness, alkalinity, conductivity, acidity, total organic carbon, and particulate matter were 144 mg/L as CaCO₃, 180 mg/L as CaCO₃, and 300 µmhos/cm, 25 mg/L phenolphthalein acidity as CaCO₃, 0.9 mg/L and 0.33 mg/L, respectively.

²Temperature measured continuously during the test ranged from approximately 21.5 to 22.5°C.

³No temperature measurements were made due to 100% mortality in the replicate.

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Table 2
Dissolved Oxygen¹ of Water in the Test Chambers

Sponsor: Shell Development Co.									
Test Substance: DAN 218									
Test Organism: Fathead Minnow (<i>Pimephales promelas</i>)									
Dilution Water: Well Water									
Nominal Concentration (mg DAN 218/L)	Replicate	Dissolved Oxygen (mg/L)							
		Day 0 (New)	Day 1 (Old)	Day 1 (New)	Day 2 (Old)	Day 2 (New)	Day 3 (Old)	Day 3 (New)	Day 4 (Old)
Control	A	8.3	7.0	8.4	7.3	8.4	7.4	8.6	7.3
	B	8.3	7.0	8.4	7.4	8.4	7.4	8.6	7.4
0.052	A	8.3	6.8	8.4	7.2	8.2	7.2	8.6	7.3
	B	8.4	6.5	8.4	7.2	8.2	7.2	8.6	7.2
0.086	A	8.4	6.2	8.5	7.1	8.2	7.0	8.6	7.1
	B	8.4	6.6	8.4	7.0	8.2	7.3	8.6	7.2
0.14	A	8.4	6.8	8.5	7.4	8.2	7.4	8.6	7.3
	B	8.4	6.8	8.5	7.4	8.2	7.2	8.6	7.2
0.24	A	8.4	6.4	8.5	7.0	8.2	7.4	8.7	7.2
	B	8.4	6.8	8.5	7.1	8.2	7.5	8.7	7.2
0.40	A	8.4	5.8	-- ²	--	--	--	--	--
	B	8.4	6.2	--	--	--	--	--	--

¹ A dissolved oxygen concentration of 5.2 mg/L represents 60% saturation at 22°C in fresh water.

² No DO measurements were made due to 100% mortality in the replicate.

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Table 3
pH of Water in the Test Chambers

Sponsor: Shell Development Co.									
Test Substance: DAN 218									
Test Organism: Fathead Minnow (<i>Pimephales promelas</i>)									
Dilution Water: Well Water									
Nominal Concentration (mg DAN 218/L)	Replicate	pH							
		Day 0 (New)	Day 1 (Old)	Day 1 (New)	Day 2 (Old)	Day 2 (New)	Day 3 (Old)	Day 3 (New)	Day 4 (Old)
Control	A	8.5	8.3	8.5	8.3	8.6	8.3	8.5	8.3
	B	8.5	8.3	8.5	8.4	8.6	8.3	8.6	8.3
0.052	A	8.5	8.3	8.5	8.4	8.6	8.4	8.6	8.3
	B	8.6	8.3	8.6	8.3	8.5	8.3	8.6	8.3
0.086	A	8.6	8.3	8.6	8.3	8.6	8.4	8.6	8.4
	B	8.5	8.3	8.5	8.3	8.6	8.4	8.6	8.3
0.14	A	8.5	8.3	8.5	8.4	8.6	8.4	8.6	8.3
	B	8.6	8.3	8.6	8.4	8.6	8.3	8.6	8.3
0.24	A	8.5	8.3	8.5	8.4	8.6	8.5	8.6	8.3
	B	8.6	8.4	8.5	8.4	8.6	8.4	8.6	8.3
0.40	A	8.6	8.2	-- ¹	--	--	--	--	--
	B	8.5	8.3	--	--	--	--	--	--

¹No pH measurements were made due to 100% mortality in the replicate.

Table 4
Cumulative Percent Mortality and Treatment-Related Effects¹

Sponsor:		Shell Development Company													
Test Substance:		DAN 218													
Test Organism:		Fathead Minnow (<i>Pimephales promelas</i>)													
Dilution Water:		Well Water													
Nominal Concentration (mg DAN 218/L)	Rep.	No Exposed	7 Hours			24 Hours			48 Hours			72 Hours			Cumulative Percent Mortality
			No. Dead	Effects	No. Dead	Effects	No. Dead	Effects	No. Dead	Effects	No. Dead	Effects			
Negative Control	A	10	0	10 AN	0	10 AN	0	10 AN	0	10 AN	0	10 AN	0	10 AN	0%
	B	10	0	10 AN	0	10 AN	0	10 AN	0	10 AN	0	10 AN	0	10 AN	
0.052	A	10	0	10 AN	0	10 AN	0	10 AN	0	10 AN	0	10 AN	0	10 AN	0%
	B	10	0	10 AN	0	10 AN	0	10 AN	0	10 AN	0	10 AN	0	10 AN	
0.086	A	10	0	10 AN	0	10 AN	0	10 AN	0	10 AN	0	10 AN	0	10 AN	0%
	B	10	0	10 AN	0	10 AN	0	10 AN	0	10 AN	0	10 AN	0	10 AN	
0.14	A	10	0	10 AN	3	7 AN	3	7 AN	3	7 AN	3	7 AN	3	7 AN	15%
	B	10	0	10 AN	0	10 AN	0	10 AN	0	10 AN	0	10 AN	0	10 AN	
0.24	A	10	0	10 AN	0	10 AN	0	10 AN	4	6 AN	8	2 AN	9	1 AN	65%
	B	10	0	10 AN	0	10 AN	0	10 AN	3	7 AN	4	6 AN	4	6 AN	
0.40	A	10	0	10 AN	10	-	10	-	10	-	10	-	10	-	100%
	B	10	1	9 AN	10	-	10	-	10	-	10	-	10	-	

¹ Observed Effects: AN = Appears Normal; - Not applicable due to total mortality.

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Table 5
LC50 Values

Sponsor:	Shell Development Company			
Test Substance:	DAN 218			
Test Organism:	Fathead Minnow (<i>Pimephales promelas</i>)			
Dilution Water:	Well Water			
Time	LC50 (mg DAN 218/L)	Lower 95% Confidence Limits (mg DAN 218/L)	Upper 95% Confidence Limits (mg DAN 218/L)	Statistical Method
24 Hours	0.31	0.24	0.40	Binomial
48 Hours	0.22	0.20	0.26	Moving Average
72 Hours	0.20	0.18	0.23	Moving Average
96 Hours	0.20	0.18	0.23	Moving Average

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APPENDIX I
Conductivity, Hardness, Alkalinity, and pH of Well Water Measured
During the 4-Week Period Immediately Preceding the Test

Sponsor: Shell Development Company
Test Substance: DAN 218
Test Organism: Fathead Minnow (*Pimephales promelas*)
Dilution Water: Well Water

	Mean	Range
Conductivity (μ mhos/cm)	313 (n = 4)	310 - 320
Hardness (mg/L as CaCO ₃)	134 (n = 4)	132 - 136
Alkalinity (mg/L as CaCO ₃)	177 (n = 4)	168 - 186
pH	8.1 (n = 4)	8.0 - 8.2

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APPENDIX II
Pesticides, Organics, Metals and Other Inorganics Analyzed
in Wildlife International Ltd. Well Water¹

Sponsor: Shell Development Company
Test Substance: DAN 218
Test Organism: Fathead Minnow (*Pimephales promelas*)
Dilution Water: Well Water

ANALYSIS	MEASURED CONCENTRATION
Organophosphorus & Organonitrogen Pesticides	
Azodrin (Methochrotophos)	< 2.88 µg/L
Bolstar	< 0.294 µg/L
Chlorpyrifos	< 0.294 µg/L
Coumaphos	< 2.96 µg/L
Demeton	< 0.282 µg/L
Diazinon	< 0.289 µg/L
Dichlorvos	< 0.286 µg/L
Dimethoate	< 0.292 µg/L
Disulfoton	< 0.278 µg/L
EPN	< 0.581 µg/L
Ethoprop	< 0.311 µg/L
Fenthion	< 0.284 µg/L
Fensulfothion	< 1.42 µg/L
Guthion (Methyl Azinphos)	< 2.94 µg/L
Malathion	< 0.298 µg/L
Merphos	< 0.306 µg/L
Mevinphos	< 0.301 µg/L
Naled	< 1.59 µg/L
Methylparathion	< 0.288 µg/L
Parathion	< 0.293 µg/L
Phorate	< 0.279 µg/L
Ronnel	< 0.294 µg/L
Stirofos	< 0.593 µg/L
Sulfotepp	< 0.284 µg/L
Tepp	< 0.302 µg/L
Tokuthion	< 0.314 µg/L
Trichloronate	< 0.300 µg/L
Metals and Other Inorganics	
Aluminum	< 40.0 µg/L
Arsenic	< 2.5 µg/L
Beryllium	< 5.0 µg/L
Cadmium	< 5.0 µg/L
Calcium	35800 µg/L
Chromium	< 5.0 µg/L
Copper	< 5.0 µg/L
Iron	< 45.0 µg/L
Lead	3.2 µg/L
Magnesium	13.6 µg/L
Manganese	15.8 µg/L
Mercury	< 0.20 µg/L
Nickel	< 10.0 µg/L
Potassium	6570 µg/L
Selenium	< 2.5 µg/L
Silver	< 5.0 µg/L
Sodium	22100 µg/L
Zinc	< 15.0 µg/L
Molybdenum	< 10.0 µg/L

¹ Analyses performed by Environmental Science & Engineering, Inc., Gainesville, Florida for samples collected on May 10, 1993.

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APPENDIX II (Continued)

Pesticides, Organics, Metals, and Other Inorganics Analyzed
in Wildlife International Ltd. Well Water¹

Sponsor: Shell Development Company
Test Substance: DAN 218
Test Organism: Fathead Minnow (*Pimephales promelas*)
Dilution Water: Well Water

ANALYSIS**MEASURED
CONCENTRATION****Miscellaneous Measurements**

Total Dissolved Solids	292 mg/L
Ammonia Nitrogen	< 0.02 mg/L
Total Organic Carbon	2.2 mg/L
Total Cyanide	< 0.001 mg/L

Organochlorines and PCBs

Aldrin	< 0.006 µg/L
Alpha BHC	< 0.006 µg/L
Beta BHC	< 0.006 µg/L
Delta BHC	< 0.006 µg/L
Gamma BHC (Lindane)	< 0.006 µg/L
Chlordane	< 0.029 µg/L
DDD, pp'	< 0.006 µg/L
DDE, pp'	< 0.006 µg/L
DDT, pp'	< 0.006 µg/L
Dieldrin	< 0.006 µg/L
Endosulfan, A	< 0.006 µg/L
Endosulfan, B	< 0.006 µg/L
Endosulfan Sulfate	< 0.006 µg/L
Endrin	< 0.006 µg/L
Endrin Aldehyde	< 0.006 µg/L
Heptachlor	< 0.006 µg/L
Methoxychlor	< 0.006 µg/L
Heptachlor Epoxide	< 0.006 µg/L
Toxaphene	< 0.588 µg/L
PCB-1016	< 0.294 µg/L
PCB-1221	< 0.294 µg/L
PCB-1232	< 0.294 µg/L
PCB-1242	< 0.294 µg/L
PCB-1248	< 0.294 µg/L
PCB-1254	< 0.294 µg/L
PCB-1260	< 0.294 µg/L

Chlorophenoxy Acid Herbicides

2,4-D, Total	< 0.020 µg/L
2,4-DB	< 0.020 µg/L
2,4,5-T Water	< 0.020 µg/L
2,4,5-TP/Silvex	< 0.020 µg/L
Dalapon	< 0.020 µg/L
Dicamba (Banvel)	< 0.020 µg/L
Dichloroprop	< 0.020 µg/L
Dinoseb	< 0.020 µg/L
MCPA	< 0.400 µg/L
MCPD	< 0.400 µg/L

¹ Analyses performed by Environmental Science & Engineering, Inc., Gainesville, Florida for samples collected on May 10, 1993.

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APPENDIX III

LC50 Calculations

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APPENDIX III

A(N) 96-H LC50 (24-H DATA) STUDY
WITH FATHEAD MINNOW

WILDLIFE INTERNATIONAL LTD. PROJECT NUMBER: 109A-111

SPONSOR NAME: Shell Development Company

TEST MATERIAL: DAN 218

CONC. mg/L	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROBABILITY (PERCENT)
.4	20	20	100	LESS THAN 0.001
.24	20	0	0	LESS THAN 0.001
.14	20	3	15	.1288413
.086	20	0	0	LESS THAN 0.001
.052	20	0	0	LESS THAN 0.001

THE BINOMIAL TEST SHOWS THAT .24 AND .4 CAN BE USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT CONFIDENCE LIMITS BECAUSE THE ACTUAL CONFIDENCE LEVEL ASSOCIATED WITH THESE LIMITS IS GREATER THAN 95 PERCENT.

AN APPROXIMATE LC50 OF .3098387 IS OBTAINED BY NONLINEAR INTERPOLATION BETWEEN .24 AND .4

WHEN THERE ARE LESS THAN TWO CONCENTRATIONS AT WHICH THE PERCENT DEAD IS BETWEEN 0 AND 100, NEITHER THE MOVING AVERAGE METHOD NOR THE PROBIT METHOD CAN GIVE ANY STATISTICALLY SOUND RESULTS.

COMPARE RESULTS WITH ORIGINAL DATA TO SEE IF THEY ARE REASONABLE

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APPENDIX III

A(N) 96-H LC50 (48-H DATA) STUDY
WITH FATHEAD MINNOW

WILDLIFE INTERNATIONAL LTD. PROJECT NUMBER: 109A-111

SPONSOR NAME: Shell Development Company

TEST MATERIAL: DAN 218

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*****
CONC.      NUMBER      NUMBER      PERCENT      BINOMIAL
mg/L       EXPOSED      DEAD        DEAD          PROBABILITY
                                     (PERCENT)
.4          20          20          100          LESS THAN 0.001
.24         20          7           35           13.15878
.14         20          3           15           .1288413
.086        20          0           0            LESS THAN 0.001
.052        20          0           0            LESS THAN 0.001
*****
```

THE BINOMIAL TEST SHOWS THAT .14 AND .4 CAN BE
USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT
CONFIDENCE LIMITS BECAUSE THE ACTUAL CONFIDENCE LEVEL
ASSOCIATED WITH THESE LIMITS IS GREATER THAN 95 PERCENT.

AN APPROXIMATE LC50 OF .2626846 IS OBTAINED BY
NONLINEAR INTERPOLATION BETWEEN .24 AND .4

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-----RESULTS CALCULATED USING THE MOVING AVERAGE METHOD-----
SPAN      G          LC50      95 PERCENT CONFIDENCE LIMITS
3          5.121859E-02 .2229305 .195536 .259578
2          8.643141E-02 .2371472 .2061045 .2726118
```

AN LC50 CALCUALTED USING THE MOVING AVERAGE METHOD MAY
NOT BE A VERY GOOD ESTIMATE IF THE SPAN IS MUCH LESS THAN
THE NUMBER OF CONCENTRATIONS

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*****RESULTS CALCULATED USING THE PROBIT METHOD*****
ITERATIONS      G          H      CHI-SQUARE      PROBABILITY
5          .1365706      1      5.57952      .1339587
```

SLOPE = 6.40194
95 PERCENT CONFIDENCE LIMITS = 4.036073 AND 8.767807

LC50 = .2341766
95 PERCENT CONFIDENCE LIMITS = .2012724 AND .2734786

COMPARE RESULTS WITH ORIGINAL DATA TO SEE IF THEY ARE REASONABLE

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APPENDIX III

A(N) 96-H LC50 (72-H DATA) STUDY
WITH FATHEAD MINNOW

WILDLIFE INTERNATIONAL LTD. PROJECT NUMBER: 109A-111

SPONSOR NAME: Shell Development Company

TEST MATERIAL: DAN 218

CONC. mg/L	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROBABILITY (PERCENT)
.4	20	20	100	LESS THAN 0.001
.24	20	12	60.00001	25.17218
.14	20	3	15	.1288413
.086	20	0	0	LESS THAN 0.001
.052	20	0	0	LESS THAN 0.001

THE BINOMIAL TEST SHOWS THAT .14 AND .4 CAN BE
USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT
CONFIDENCE LIMITS BECAUSE THE ACTUAL CONFIDENCE LEVEL
ASSOCIATED WITH THESE LIMITS IS GREATER THAN 95 PERCENT.

AN APPROXIMATE LC50 OF .2145719 IS OBTAINED BY
NONLINEAR INTERPOLATION BETWEEN .14 AND .24

-----RESULTS CALCULATED USING THE MOVING AVERAGE METHOD-----

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
3	5.032284E-02	.2035348	.1788505	.234199
2	8.643143E-02	.2100157	.179397	.2400493
1	.439513	.2145719	.1793606	.3286224

AN LC50 CALCULATED USING THE MOVING AVERAGE METHOD MAY
NOT BE A VERY GOOD ESTIMATE IF THE SPAN IS MUCH LESS THAN
THE NUMBER OF CONCENTRATIONS

*****RESULTS CALCULATED USING THE PROBIT METHOD*****

ITERATIONS	G	H	CHI-SQUARE	PROBABILITY
6	.1366666	1	1.251392	.7407064

SLOPE = 7.061529
95 PERCENT CONFIDENCE LIMITS = 4.450991 AND 9.672066

LC50 = .2078551
95 PERCENT CONFIDENCE LIMITS = .1797952 AND .2404512

COMPARE RESULTS WITH ORIGINAL DATA TO SEE IF THEY ARE REASONABLE

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APPENDIX III

A(N) 96-H LC50 (96-H DATA) STUDY
WITH FATHEAD MINNOW

WILDLIFE INTERNATIONAL LTD. PROJECT NUMBER: 109A-111

SPONSOR NAME: Shell Development Company

TEST MATERIAL: DAN 218

CONC. mg/L	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROBABILITY (PERCENT)
.4	20	20	100	LESS THAN 0.001
.24	20	13	65	13.15878
.14	20	3	15	.1288413
.086	20	0	0	LESS THAN 0.001
.052	20	0	0	LESS THAN 0.001

THE BINOMIAL TEST SHOWS THAT .14 AND .4 CAN BE
USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT
CONFIDENCE LIMITS BECAUSE THE ACTUAL CONFIDENCE LEVEL
ASSOCIATED WITH THESE LIMITS IS GREATER THAN 95 PERCENT.

AN APPROXIMATE LC50 OF .2059399 IS OBTAINED BY
NONLINEAR INTERPOLATION BETWEEN .14 AND .24

-----RESULTS CALCULATED USING THE MOVING AVERAGE METHOD-----

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
3	5.014341E-02	.1998325	.1755949	.2294872
2	.0864314	.2048784	.1741763	.2341963
1	.3590504	.20594	.1747937	.2764482

AN LC50 CALCULATED USING THE MOVING AVERAGE METHOD MAY
NOT BE A VERY GOOD ESTIMATE IF THE SPAN IS MUCH LESS THAN
THE NUMBER OF CONCENTRATIONS

*****RESULTS CALCULATED USING THE PROBIT METHOD*****

ITERATIONS	G	H	CHI-SQUARE	PROBABILITY
6	.1395682	1	.8233643	.8438708

SLOPE = 7.294633
95 PERCENT CONFIDENCE LIMITS = 4.569444 AND 10.01982

LC50 = .2028991
95 PERCENT CONFIDENCE LIMITS = .1758623 AND .2342184

COMPARE RESULTS WITH ORIGINAL DATA TO SEE IF THEY ARE REASONABLE

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APPENDIX IV

CHANGES TO PROTOCOL

The study was conducted in accordance with the approved Protocol with the following changes:

1. The proposed dates, study room, test concentrations, test substance number and receipt date were amended to the protocol.
2. The protocol was amended to change the sample volume, preparation and shipment.
3. Water and stock samples for analytical confirmation were collected in sample containers composed of flint glass.
4. Analytical measurements were not performed to confirm exposure concentrations.

In the opinion of the Study Director, the above changes in the approved protocol did not adversely affect the results of this study.

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APPENDIX V
PERSONNEL INVOLVED IN THE STUDY

The following key personnel were involved in the conduct or management of this study:

Aquatic Toxicology Laboratory

1. James P. Swigert, Ph.D., Manager, Aquatic Toxicology
2. Kurt R. Drottter, Senior Aquatic Biologist
3. Barry P. Conner, Aquatic Biologist
4. Gordon R. Pepper, Aquatic Biologist
5. Cynthia A. Roberts, Senior Aquatic Biologist
6. Edward C. Schaefer, Manager, Biodegradation

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